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The influence of sample volume applied to the Makler sperm counting chamber upon the measured concentration of latex beads: A multi-centre study

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ABSTRACT

Objective: To undertake a multi-centre study to maximize the number of Makler chambers used. **Methods:** A total of 15 laboratories participated with 31 Makler chambers. A suspension of latex beads was prepared to a concentration of 20 millions per milliliter, and 0.5 mL aliquots distributed to each participating laboratory. They measured the concentration on their Makler chamber(s) used for routine semen analysis by adding 3, 4, 5, 7 and 10 μL volumes of bead suspension to the chamber. **Results:** There was no difference in within-chamber analysis of the bead concentration according to the volume of bead suspension applied within the range of 3-10 μL ($F_{4,14}$=2.634, P=0.056). However, the between-chamber effects were significantly different ($F_{30,124}$=4.937, P=0.000), and 24/31 (77.5%) chambers tested had an average bias>10% compared to the target bead concentration. **Conclusions:** A volume of 3-10 μL added to Makler counting chambers does not influence the concentration measured of latex beads, but the between-chamber variability and positive bias seen would suggest that other sources of error are present which are yet to be identified.

1. Introduction

Makler chambers have been designed specifically for the measurement of the concentration of human sperm in semen[1,2], and are popular, being used by approximately 25% of clinical laboratories enrolling in external quality assurance schemes for semen analysis[3,4]. Following the initial validation against haemocytometers[1] and subsequent confirmation of the good performance of the Makler chamber[5-7], others have found the Makler to overestimate sperm concentration and give poor precision[8,9]. In search of a source of error which may explain instances of poor performance, a formal investigation of the effect of sample volume was prompted by the manufacturer's instructions

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that "a small, uncalibrated drop from a well-mixed undiluted specimen is placed in the center of the Chamber by means of a simple rod and immediately covered". A multi-centre study was undertaken to maximize the number of Makler chambers used.

2. Materials and methods

A suspension of 6.4 μm diameter latex beads suspended in a colloidal fluid based on 4% modified gelatin extract (Gelofusine; B Braun Australia, Bella Vista, NSW 2153, Australia) was prepared to a concentration of 20 millions per milliliter by EQASRM (External Quality Assurance Schemes for Reproductive Medicine, PO Box 162, Northlands, Western Australia 6905, Australia) and verified using an improved Neubauer haemocytometer[10]. Aliquots of 0.5 mL contained within 3 mL cryovials (ProScitech) were distributed to each participating laboratory. Laboratories were blinded to the bead concentration. After adequate resuspension of the beads, each laboratory measured the concentration on the Makler chambers (Sefi Medical Instruments, Haifa, Israel) used for routine semen analysis in their laboratory. Laboratories were instructed to count the number of beads in the whole grid to minimize counting errors. Analysis of within- and between-laboratory effects were made by repeated measures ANOVA using StatistiXL (Nedlands, Western Australia 6009, Australia), an add-in programme for Excel 2003 (Microsoft Corporation). Differences were considered significant if $P<0.05$.

3. Results

The results are summarized in Table 1. There was no difference in within-chamber analysis of the bead concentration according to the volume of bead suspension applied within the range of 3-10 μL ($F_{4,14}=2.634$, $P=0.056$). However, the between-chamber effects were significantly different ($F_{30,124}=4.937$, $P=0.000$) as indicated by the large range of values. Of the 31 chambers tested, 24 (77.5%) tested had an average bias greater than 10% compared to the target bead concentration.

Table 1
Concentration of latex beads according to volume of sample applied to the Makler chamber.

Volume added to chamber (μL)	Bead concentration ($\times10^6$/mL)	
	Mean±SEM	Range
3	23.7±1.0	17.4-42.0
4	26.4±1.2	18.3-39.2
5	25.3±0.9	16.2-37.4
7	25.2±1.2	13.0-41.0
10	26.0±1.2	16.4-41.0

A total of 31 chambers were used from 15 laboratories, and the bead suspension had a target concentration of 2×10^7/mL.

4. Discussion

The results of this study do not indicate a difference in concentration with regards to volume applied to the chamber within the range of 3-10 μL. It does however show an increase in the mean concentration determined compared to the target bead concentration with more than three quarters of the chambers tested having an average bias>10%. This is consistent with an overestimation of sperm concentration found elsewhere when using the Makler chamber[11,12], which may be associated with wear and tear of the instruments[13]. However, the minimal variation in concentration measured by the same Makler chambers indicate good reproducibility of results rather than poor precision as found in some other studies[8,9]. This present study has shown the effectiveness of the use of multicentre studies whereby the involvement of a large number of participants allowed comparisons both within- and between-chamber to be made on a number of chambers far in excess of that found in single locations.

In summary, a volume of 3-10 μL added to a Makler counting chamber does not influence the concentration measured. The between-chamber variability and positive bias seen would suggest that other sources of error are present which are yet to be identified. Further work is required to determine the factors which are important in adversely influencing the performance of the Makler chamber.

Conflict of interest statement

We declare that we have no conflict of interest.

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References

[1] Makler A. A new chamber for rapid sperm count and motility estimation. *Fertil Steril* 1978; **30**(3): 313-318.
[2] Makler A. The improved ten-micrometer chamber for rapid sperm count and motility evaluation. *Fertil Steril* 1980; **33**(3): 337-338.
[3] Keel B, Quinn P, Schmidt CJ, Serafy NJ, Serafy NS, Schalue T. Results of the American Association of Bioanalysts national proficiency testing programme in andrology. *Hum Reprod* 2000; **15**(3): 680-686.
[4] Matson P. External quality assessment for semen analysis and sperm antibody detection: results of a pilot scheme. *Hum Reprod* 1995; **10**(3): 620-625.
[5] Cardona-Maya W, Berdugo J, Cadavid A. Comparing the sperm concentration determined by the Makler and the Neubauer chambers. *Actas Urol Esp* 2008; **32**(4): 443-445.
[6] Keel B, Vanderslice W. Comparison of sperm counting chambers used by participants in a national andrology proficiency testing program. *Fertil Steril* 2004; **82**(Suppl 2): S329-330.
[7] Mahmoud A, Depoorter B, Piens N, Comhaire F. The performance of 10 different methods for the estimation of sperm concentration. *Fertil Steril* 1997; **68**(2): 340-345.
[8] Bailey E, Fenning N, Chamberlain S, Devlin L, Hopkisson J, Tomlinson M. Validation of sperm counting methods using limits of agreement. *J Androl* 2007; **28**(3): 364-373.
[9] Christensen P, Stryhn H, Hansen C. Discrepancies in the determination of sperm concentration using Bürker-Türk, Thoma and Makler counting chambers. *Theriogenology* 2005; **63**(4): 992-1003.
[10] Zuvela E, Junk S, Moska N, Matson P. The use of latex beads in external quality assurance and internal quality control for routine semen analysis. *Reprod Biol* 2011; **11**(3): 264-275.
[11] Coetzee K, Menkveld R. Validation of a new disposable counting chamber. *Arch Androl* 2001; **47**(2): 153-156.
[12] Seaman E, Goluboff E, BarCharma N, Fish III H. Accuracy of semen counting chambers as determined by the use of latex beads. *Fertil Steril* 1996; **66**(4): 662-665.
[13] Tzanakaki D, Baka S, Hassiakos D, Konidaris S. The fair wear and tear of time on a Makler counting chamber can affect the accuracy of assessing sperm concentration. *Hum Reprod* 2007; **22**(Suppl 1): i165.